REMARKS

Support for the new claims is found in priority document U.S. Application No. 09/031,629, as indicated by paragraph number therein.

I) Support for claim 76, which features the treatment of autoimmune by initiating a signaling pathway that activates the transcription factor NFκB is as follows.

> [0013] As described below, ubiquitination affects signal transduction, as it may mark certain cell-surface growth-factor receptors for endocytosis and destruction; further, it is known that ubiquitination, coupled with phosphorylation, stimulates the signaling pathway that activates the transcription factor NFκB. Ubiquitin also plays a role in protein degradation pathways regulating cell differentiation and death during development.

> [0020] Proteins that control cell-cycle progression may respond to environmental cues, such as are provided by growth factors. Growth factor-stimulated signaling pathways are, themselves controlled in part by ubiquitination. One of the best studied examples is the NFκB pathway (see below). Binding of the cytokine tumor necrosis factor-.alpha. (TNF-α) to cell-surface receptors, or the occurrence of another proinflammatory or stress event (e.g. hypoxia), initiates a signaling cascade that activates NFκB (see below) and c-Jun, transcription factors that govern the proliferative response in cells.

II) Support for claims 77, 78, and 82 which feature compounds that stimulate the signaling pathway of claims 76 and 81 is as follows:

> [0020] Proteins that control cell-cycle progression may respond to environmental cues, such as are provided by growth factors. Growth factor-stimulated signaling pathways are, themselves controlled in part by ubiquitination. One of the best studied examples is the NFκB pathway (see below). Binding of the cytokine tumor necrosis factor-.alpha. (TNF-α) to cell-surface receptors, or the occurrence of another proinflammatory or stress event (e.g. hypoxia), initiates a signaling cascade that activates NFκB (see below) and c-Jun, transcription factors that govern the proliferative response in cells.

[0161] The invention contemplates methods of treating autoimmunity by restoring proteolytic processing, based upon the observation that NFκB activity is absent in the NOD mouse model of autoimmune disease. Restoration of proteolytic processing, such as would result in the restoration of NFκB activity, may be directed at the proteasome, the ubiquitinating machinery or protein kinases.

[0164] The invention contemplates methods of treating autoimmunity by restoring proteolytic processing by blocking the activity of inhibitors of proteasome function or changing the specificity of a proteasome subunit to favor activation of the substrate(s) deficient in an autoimmune disease, so that correct protein processing is restored.

[0165] Inhibition of proteasome activity blocks the production of activated NFκB and other essential proteins, as described above; therefore, in order to promote correct protein processing, it may be necessary to inactivate cellular inhibitors of the proteasome. Such endogenous inhibitors of proteasome activities have been isolated. These include the 240 kD and the 200 kD inhibitors isolated from human erythrocytes (Murakarni et al., 1986, Proc. Natl. Acad. Sci. U.S.A., 83: 7589-7592; Li et al., 1991, Biochemistry, 30: 9709-9715) and purified CF-2 (Goldberg, 1992, Eur. J. Biochem., 203: 9-23).

[0166] Endogenous proteasome inhibitors may be inactivated by methods known in the art, which methods include the administration of antibodies which bind them specifically, the use of antisense RNA or ribozymes directed against the mRNAs which encode them (see below). Antibodies against numerous proteins are now publicly available, both through commercial and non-profit suppliers (e.g. ATCC); however, antibodies of use in the invention may, if necessary, be prepared as described below.

[0177] In the case in which NFκB activity is reduced or absent due to an `upstream` defect (that is, one involving activation by the proteasome, instead of- or in addition to a mutation in the NFκB gene itself), it is possible to circumvent the need for proteolytic activation of NFκB by introducing a constitutively-active version of the protein, such as one in which the IκB recognition site has been mutated such that IκB can no longer bind to- and inactivate NFκB. Binding of NFκB to IκB occurs through ankyrin repeats (as reviewed by Siebenlist et al., 1994, Ann. Rev. Cell. Biol., 10: 405-455); it is contemplated that sequences encoding these repeats be deleted or mutated in an NFκB subunit p100 or p105 gene

expression construct such that binding to IκB is significantly impaired or is eliminated. As a transcription/signalling factor which remains active when it is no longer required may have undesirable consequences, particularly in the absence of proteolytic which would normally inactivate it under such circumstances, administration of such a protein in limited doses or of a gene encoding it under a tightly-regulated (i.e. inducible, rather than constitutive, promoter) may be necessary. Alternatively, such a protein may be expressed at all times, provided that an inhibitor thereof is co-administered; such an inhibitor may be an antibody directed against the protein, or an antisense RNA or ribozyme directed against the message encoding it, as described below.

[0178] Inactivation of IκB may also be performed by methods described below, such as by the use of antibodies directed against it or of antisense RNA or ribozymes directed against the mRNA transcript encoding it. Preferably, such inactivation is transient, as it would otherwise lead to constitutive activation of NFκB, which activation is not, itself, normal.

III. Support for claim 80, which features autoimmune diseases treated by the method of claim 76 is as follows:

[0031] Examples of autoimmune diseases include, but are not limited to, diabetes, rheumatoid arthritis, multiple sclerosis, lupus erythematosis, myasthenia gravis, scleroderma, Crohn's disease, ulcerative colitis, Hashimoto's disease, Graves' disease, Sjogren's syndrome, polyendocrine failure, vitiligo, peripheral neuropathy, graft-versus-host disease, autoimmune polyglandular syndrome type I, acute glomerulonephritis, Addison's disease, adult-onset idiopathic hypoparathyroidism (AOIH), alopecia totalis, amyotrophic lateral sclerosis, ankylosing spondylitis, autoimmune aplastic anemia, autoimmune hemolytic anemia, Behcet's disease, Celiac disease, chronic active hepatitis, CREST syndrome, dermatomyositis, dilated cardiomyopathy, eosinophilia-myalgia syndrome, epidermolisis bullosa acquisita (EBA), giant cell arteritis, Goodpasture's syndrome, Guillain-Barre syndrome, hemochromatosis, Henoch-Schonlein purpura, idiopathic IgA nephropathy, insulin-dependent diabetes mellitus (IDDM), juvenile rheumatoid arthritis, Lambert-Eaton syndrome, linear IgA dermatosis, myocarditis, narcolepsy, necrotizing vasculitis, neonatal lupus syndrome (NLE), nephrotic syndrome, pemphigoid, pemphigus, polymyositis, primary sclerosing cholangitis, psoriasis, rapidly-progressive

glomerulonephritis (RPGN), Reiter's syndrome, stiff-man syndrome and thyroiditis.

IV. Support for claim 81, which features a method in which NFkB activity is assessed before administration of a substance to treat an autoimmune disease is as follows:

> [0067] Another aspect of the present invention is a method of detecting autoimmune disease in a mammal, comprising providing a biological sample from a mammal and detecting NFκB activity, wherein a reduction in NFκB activity from a basal state is indicative of autoimmune disease.
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> [0068] As defined herein with regard to NFκB activity, the term "reduction" refers to a loss of the ability of NFκB to direct the transcription of genes whose cis-regulatory sequences comprise an NFκB recognition site, wherein such a site is normally bound and transcription of the gene activated by NFκB. Preferably, such a reduction is in the range of 5-10% of the basal state level of activity, more preferably 25-50% and most preferably 70-100%.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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